## Claims

1. A method for characterizing samples having units, by monitoring fluctuating intensities of radiation emitted, scattered and/or reflected by said units in at least one measurement volume, the monitoring being performed by at least one detection means, said method comprising the steps of:

   a) measuring in a repetitive mode a number of photon counts per time interval of defined length,
   b) determining a function of the number of photon counts per said time interval,
   c) determining a function of specific brightness of said units on basis of said function of the number of photon counts.

2. A method according to claim 1, wherein said function of the number of photon counts per said time interval and/or said function of specific brightness is a distribution function.

A 3. The method according to ~~claim 1 and/or 2~~ claim 1, wherein said units are molecules, macromolecules, dyes, molecular aggregates, complexes, vesicles, cells, viruses, bacteria, beads, centers, or mixtures thereof in solids, liquids or gases.

A 4. The method according to ~~at least one of the claims 1 to 3~~ claim 1, wherein said units can be grouped into species which can be distinguished by their specific brightness.

A 5. A method according to ~~at least one of the claims 1 to 4~~ claim 1, wherein at least one species is luminescent, preferably fluorescent, and/or is luminescently labelled.

A 6. A method according to ~~at least one of the claims 1 to 5~~ claim 1, wherein the luminescence properties of the units are varied by conjugating them with a first molecule, in particular biotin, which binds a luminescently labelled second molecule, in particular luminescently labelled avidin or streptavidin, or vice versa.

7. A method according to claim 6 wherein the first molecule is a (6xHis)tag and the second molecule is a luminescently labelled Ni-NTA-derivatives.

A 8. A method according to ~~at least one of the claims 1 to 7~~ claim 1, wherein the luminescence properties of a unit are changed by energy transfer, in which energy absorbed by said unit is transferred upon close contact to a luminophore of an acceptor and subsequently emitted.

A 9. A method according to ~~at least one of the claims 1 to 8~~ claim 1, wherein said units each carry a number of binding sites for luminescent units.

A 10. A method according to ~~at least one of the claims 1 to 9~~ claim 1, wherein the measurement volume is only a part of the total volume of the sample and has a volume $\leq 10^{-12}$ l, preferably $\leq 10^{-14}$ l.

A 11. A method according to ~~at least one of the claims 1 to 10~~ claim 1, wherein said units are diffusing and/or being actively transported into and out of said measurement volume and/or the sample is actively transported and/or optically scanned.

A 12. A method according to ~~at least one of the claims 1 to 11~~ claim 1, wherein the measurement volumes are arranged on a two-dimensional carrier, in particular on a membrane or in sheets having wells, or in linear way, preferably in a capillary system.

13. A method according to ~~at least one of the claims 1 to 12~~ claim 1, wherein a confocal microscope set-up is used, comprising at least one microscope objective, preferably with a numerical aperture ≥ 0.9, for both focussing an incident laser beam and collecting radiation emitted, scattered and/or reflected by said units of said sample, a dichroic mirror, a pin-hole in the image plane of said microscope objective, a detection means, a data acquisition means, and optionally means for scanning and/or actively transporting said sample.

14. A method according to ~~at least one of the claims 1 to 13~~ claim 1, wherein said measurement volume is restricted by the use of elements of near field optical microscopy, or their combination with conventional microscopy optics.

15. A method according to ~~at least one of the claims 1 to 14~~ claim 1, wherein fluorescence is induced using multiple photon excitation.

16. A method according to ~~at least one of the claims 1 to 15~~ claim 1, wherein the parameters of a spatial brightness function characteristic for the optical set-up are determined by measuring numbers of photon ~~counts~~ per defined time intervals in a repetitive mode from radiation emitted, scattered and/ or reflected by a single species.

17. A method according to ~~at least one of the claims 1 to 16~~ claim 1, wherein the dimension and two-dimensional shape of the pinhole positioned in the focal plane of the microscope is used as a modelling parameter of the spatial brightness function.

18. A method according to ~~at least one of the claims 1 to 17~~ claim 1, wherein the convergence angle of the incident laser beam is used as a modelling parameter of the spatial brightness function.

A 19. A method according to ~~at least one of the claims 1 to 18~~ claim 1, wherein the concentration and/or specific brightness of at least one species of said units is determined.

A 20. A method according to ~~at least one of the claims 1 to 19~~ claim 1, wherein said distribution of the number of photon counts is fitted using ~~a priori information~~ on the sample.

A 21. A method according to ~~at least one of the claims 1 to 20~~ claim 1, wherein said distribution of the number of photon counts is processed by applying an inverse transformation with linear regularization and/or constraints.

A 22. A method according to ~~at least one of the claims 1 to 21~~ claim 1, wherein the experimental parameters of the detection means, in particular dead time and afterpulsing probability of the detection means, are determined by measuring number of photon counts per defined time interval in a repetitive mode while the detection means is exposed to light of constant intensity or high frequency laser pulses.

A 23. A method according to ~~at least one of the claims 1 to 22~~ claim 1, wherein background count rate of the equipment is determined.

A 24. A method according to ~~at least one of the claims 1 to 23~~ claim 1, wherein the length of said time interval is in average smaller than the characteristic correlation time of radiation intensity fluctuations.

A 25. A method according to ~~at least one of the claims 1 to 24~~ claim 1, wherein the length of said time interval is selected to yield in average more than one, preferably one to ten, photon counts per said unit.

A 26. A method according to ~~at least one of the claims 1 to 25~~ claim 1, wherein the concentration of the sample or the size of the

measuring volume is selected to have in average no more than a few, preferably about one unit per measurement volume.

A 27. A method according to ~~at least one of the claims 1 to 26~~ claim 1, wherein at least one individual unit is statistically analyzed in terms of its specific brightness which may fluctuate or change non-stochastically.

A 28. A method according to ~~at least one of the claims 1 to 27~~ claim 1 for use in high throughput screening, diagnostics, monitoring of polymerization, aggregation and degradation processes, or analytics of nucleic acids.